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(54) Title: USE OF METALLOPORPHYRINS TO POTENTIATE AIDS THERAPY

(57) Abstract

Methods and compositions for treating viral infections, especially retroviral infections such as AIDS comprising administration of a heme product in pharmaceutical compositions which may additionally contain an anti-retroviral drug such as AZT with or without erythropoietin.

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USE OF METALLOPORPHYRINS TO POTENTIATE AIDS THERAPY

RELATED APPLICATION

This aplication is a continuation in part application of commonly owned copending patent application serial number 07/560,191.

BACKGROUND OF THE INVENTION

This invention is concerned with methods and compositions for treating viral infections such as retroviral infections in mammals, especially humans.

The retroviruses are a broad group of RNA viruses which during their replication employ the reverse transcription enzyme (RT) to convert an RNA message to DNA. The retroviridae family of viruses includes Lentiviruses (visna, maedi, progressive pneumonia virus - "slow viruses"), Spumaviruses (foamy viruses) and Oncornaviruses (types A, B, C, D, RNA tumor viruses). The retroviruses have been shown to infect murine, avian, feline, primate, and human species.

The human immunodeficiency virus (HIV-1) or human T-cell lymphotropic virus (HTLV-III) which causes Acquired Immune Deficiency Syndrome (AIDS), AIDS related complex (ARC) and AIDS related diseases is a retrovirus. Also, the feline leukemia virus (Fe.) of cats is a retrovirus.

Retroviruses of the HIV species such as HIV 1 and HIV 2 are cytopathic for helper/inducer T cells in vitro. The HIV virus is the etiologic agent of the acquired immune deficiency syndrome (AIDS) and related diseases. To date, thousands of cases of AIDS have been reported in

the United States, and the incidence and prevalence of this disease continues to increase. During 1989 alone, 35,238 cases of AIDS were reported to the Centers for Disease Control with a total of more than 100,000 cases since 1981.

AIDS is almost always fatal within 1 to 2 years of the first clinical manifestations of illness. This disease was initially described and characterized in four high-risk groups (homosexual men, hemophiliacs, Haitians, and intravenous drug abusers); however, individuals belonging to no apparent high-risk groups have also developed the disease. AIDS is generally spread by intimate sexual contact or by the administration of infected blood products, and occasionally by the maternal-fetal route. Many patients who develop AIDS are asymptomatic when they transmit their disease to contacts because a 6-month to 5-year (or more) latency interval may exist between infection and clinical manifestations of illness.

Historically, nucleosides have been among the best anti-retroviral drugs for treating DNA and RNA viral infections such as retroviral infections. For the treatment of AIDS, 3-azido-3'-deoxythymidine (AZT) has been the most successful of these types of therapeutic agents. However, because of the known deficiencies of AZT a large number of other drugs most of which are not nucleosides are currently under study both <u>in vitro</u> and <u>in vivo</u>. Several of them have reached the stage of clinical trials. These include:

dextran sulfate	AS 101
castanospermine	Ampligen
interferons	GM-CSF
ribavirin	interleukin-2
doxorubicin	foscarnet
AL 721	suramin
Ansumycin	nefabutin

HPA-29

The use of AZT and related nucleosides is described in detail in U.S. Patent 4,724,232, the disclosure of which is incorporated herein by reference.

Antiviral therapy for the treatment of AIDS is based on the assumption that continued retroviral replication is involved in both the pathogenesis and progression of the disease. Therefore, RT has been a major target for antiviral therapy in AIDS, and indeed, most of the agents now being investigated, including AZT, act on this enzyme. The phosphorylated forms of these compounds inhibit HIV replication by acting as chain terminators. Reverse transcriptase of HIV is much more susceptible to the inhibitory effects of these phosphorylated dideoxynucleotides than mammalian DNA polymerases. Administration of AZT has been shown to result in immunologic improvements and to confer a survival advantage in patients with AIDS.

The successful treatment of AIDS with AZT has, however, been limited due to its serious adverse effects including macrocytosis anemia, neutropenia and

thrombocytopenia. AZT suppresses the proliferation of erythroid, granuclocyte, macrophage and primitive hematopoietic stem cells in a dose-related and time-dependent fashion. AZT regimens are associated with significant bone marrow toxicity. In addition, long term treatment with AZT may create a selective pressure which affords replication advantage to viruses of drug-resistant phenotype. Such variants have been isolated from patients suffering from advanced HIV-1 associated disease, sometimes, as early as six months after initiation of treatment.

Heme or ferriprotoporphyrin IX is a red pigment comprised of four subunits called pyrroles; these subunits are chemically joined to form a single large tetrapyrrole (porphyrin) ring structure. A metal atom is chelated at the center of this porphyrin: in higher organisms this metal is iron and the porphyrin ring structure is called protoporphyrin IX. In physiological systems heme is bound to certain proteins; these hemoproteins bind oxygen at the site of the metal atom or they function as components of membrane bound electron transport systems. Cellular respiration, energy generation and chemical oxidations are dependent on these heme proteins. Hemin is the chloride salt of heme. Heme synthesis and heme degradation are critical to the maintenance of cellular heme homeostasis and hematopoietic differentiation.

Tsutsui and Mueller (1) reported that the virion-associated RT activity of Rauscher murine leukemia virus was inhibited by hemin at a concentration of 100um. Hemin

is the chloride of ferriprotoporphyrin IX (heme). The inhibition of RT by this large concentration of hemin was reversible and appeared to be directed against the enzyme rather than the template. On the other hand, hemin did not inhibit the activity of RT purified from avian myeloblastosis virus.

Synthetic hemes include a wide variety of organometallic porphyrins in which the chelated atom is a metal other than iron such as tin, chromium, cobalt, zinc or manganese or analogous compounds in which the porphyrin ring structure is modified as in protoporphyrins or mesoporphyrins. Typical synthetic hemes which might be mentioned by way of example are tin protoporphyrin (SnPP), tin mesoporphyrin (SnMP), tin diiododeuteroporphyrin (SnI_2DP) and the corresponding zinc, chromium, manganese and cobalt compounds all of which are known or can be prepared by known procedures. All are useful in the practice of this invention. Tin protoporphyrin and tin mesoporphyrin are preferred because they are readily available and especially active.

Other heme derivatives useful in the practice of the invention include acid addition salts of heme, particularly amino acid salts of heme including L-amino acids such as arginine. Heme arginate is especially preferred for use in the invention, although other non-toxic acid addition salts of inorganic and organic acids especially the naturally occurring L-amino acids may also be employed.

Because of the aforesaid disadvantages of AZT and AZT type nucleosides in the treatment of retroviral infections, much time, effort and money has been expended to find replacement drugs, or at least drugs which will enhance the antiviral effects of the known therapeutic agents so as to permit lower doses, to reduce toxic sequellae or permit use of the therapeutic agents for longer periods.

BRIEF SUMMARY OF THE INVENTION

It has now been discovered that heme, synthetic hemes and heme acid addition salts either alone or coadministered with anti-HIV drugs are useful for the treatment of AIDS. For convenience, heme, heme acid addition salts and synthetic hemes will sometimes hereinafter be referred to as "heme products." Heme products will enhance the activity of antiviral compounds especially antiretroviral compounds such as AZT. The invention is particularly useful with therapeutic agents of the nucleoside type such as AZT or dideoxyinosine (DDI). The invention also includes within its scope pharmaceutical compositions containing one or more heme products, an anti-HIV infection drug such as AZT together with erythropoietin (EPO). It has been observed that the combination of heme products and EPO is effective to reverse the adverse effects of the anti-HIV drug.

The invention therefore comprises methods and compositions for the treatment of retroviral infections of mammals especially humans in need of such treatment. The invention is especially applicable to controlling AIDS infections of humans.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1, 2, 3 4 and 5 illustrate by means of graphs the results of studies described herein utilizing hemin, AZT, and combinations of hemin and AZT, and combinations of these agents with EPO.

DETAILED DESCRIPTION OF THE INVENTION

As used in this description, the term "coadministration" means that the therapeutic agents are administered simultaneously or sequentially at time intervals sufficiently close so that the administration of one agent has a beneficial effect on the action of the other. Normally, they will be administered parenterally in one dosage unit although the invention is not so limited. In fact, the one component of the therapeutic mixture, e.g. the nucleoside can be administered orally and the heme product parenterally.

For convenience, the invention will be described principally as it relates to heme, AZT and EPO, but as is clear from the foregoing, the invention is not limited to these materials.

A number of tests were conducted to determine the antiviral efficacy of heme alone or with various concentrations of AZT utilizing an AZT sensitive strain of HIV, an AZT resistant strain of HIV, both isolated from human patients, and AZT sensitive strain HTLV-III B obtained from ERC Bioservices Corporation.

Other tests were conducted to establish the ability of the combination of heme products and EPO to reverse the adverse effects of anti-retroviral drugs such as AZT.

Cells and viruses

In this study of heme products coadministered with AZT a total of three strains of HIV-I were utilized. Two strains were isolated (2) from blood obtained from AIDS patients. One isolate was obtained from a patient who had been on AZT therapy for four months; this isolate is defined as AZT-resistant (at 1uM AZT), in Table 1. The other isolate was derived from a patient who had never received AZT and is referred to as AZT-sensitive (at 1uM AZT) in Table 1. The third HIV strain (HTLV-IIIB) was obtained from ERC Bioservices Corporation (Rockville, MD) through the NIAID AIDS Research and Reference Reagent Program.

HTLV-IIIB (3, 4) was replicated in the H9 cell line (5, 6) (ERC Bioservices Corp.) The AZT-resistant patient-derived HIV was also adapted to the H9 cell line but the AZT-sensitive HIV (Table 1) could not be adapted

to these cells. The two HIV strains were passaged in H9 cells in IMDM (Gibco) supplemented with 10% fetal bovine serum (FBS) (Gibco) and subcultured at 3-4 days.

Infection of H9 with HIV

H9 cells (0.5 x 10^6/ml) were infected with the two strains of HIV (HTLV-IIIB and AZT-resistant patient isolate). Graded concentrations of hemin (Sigma, St. Louis) (0-15.0uM) were added to the two groups of cultures. The cultures were maintained in RPMI 1640 media supplemented with 10% FBS at 37°C with replacement of 50% media containing hemin twice during 7 days. An aliquot of 0.5ml was collected with an equal volume of FBS at day 7 and frozen at -70°C until assayed.

p24 assay

Samples were assayed in duplicate for the HIV antigen, p24, with the Abbott HIV antigen detection system according to the Abbott protocol (7). Control (200ul) or diluted sample (180ul) was added with 20 ul Triton X-100 to each well, followed by a polystyrene bead coated with human antibody to HTLV-IIIB. The assay plates were held at room temperature for 16-20 hours. After washing the beads with water, rabbit antibody to HTLV-III (200ul) was added to every well and the assay plates incubated at 40°C for 4 hours. After washing the beads, 200ul of goat anti-rabbit IgG conjugated with horseradish peroxidase was added to each well and the assay plates incubated at 40°C for two hours. After a final wash, the beads were

transferred from assay plates to tubes and 300ul freshly prepared OPD (o-phenylenediamine.2HCl) substrate solution with 0.02% hydrogen peroxide added to each assay tube containing one bead. The assay tubes were incubated at room temperature in the dark for 30 minutes. One ml of IN sulfuric acid was added to each tube to stop the reaction. The absorbance of controls and treated cultures was determined spectrophotometrically at 492nm.

Cultiviation of HIV in Peripheral Blood Lymphocytes (PBL)

Human PBLs ($0.5x10^6$/ml) from healthy donors were infected in vitro with HIV isolated from two AIDS patients (Table 1). AZT (Burroughs Wellcome) was added to the cultures at a final concentration of 1uM, while hemin was added at a final concentration of 1uM or 10uM in a total volume of 2ml. The cultures were maintained at 37^oC with a replacement of 50% of the media containing AZT, hemin or a combination of AZT and hemin twice within 7 days. Aliquots were collected with an equal volume of FBS on day 7 and frozen at -70^oC until assayed.

Cell Viability

Cell viability was assessed by the trypan blue exclusion method. In all experiments reported in this disclosure, cell viability was greater than 72%.

In the experiments shown in Table 1, AZT (1uM) completely inhibited HIV replication in the drug-sensitive strain (Table 1, patient 1) on day 7 in in vitro cultures

of PBL. Hemin did not alter the antiviral action of AZT when administered concurrently with the drug. Further, hemin alone (10uM) had a substantial inhibitory effect on viral replication. For the AZT-resistant HIV-isolate (Table 1, patient 2), hemin alone or AZT, in the concentrations studied had no antiviral activity. However, when a combination of AZT (1uM) and hemin (1uM or 10uM) was used, HIV replication was inhibited completely.

To extend these observations, additional experiments with hemin and AZT were carried out to study HIV replication in the H9 cell line. In these experiments the AZT-resistant HIV from patient 2 was adapted to H9 cells. HTLV-IIIB was also replicated in the H9 cell line and served as the AZT sensitive strain. It did not prove possible to adapt the AZT-sensitive HIV isolate from patient 1 to the H9 cells. H9 cells were infected with the two strains of HIV and graded amounts (0-1uM) of AZT were added to the two groups of cultures. The cultures were maintained at 37°C in RPMI 1640 supplemented with 10% FBS with replacement of 50% of media containing hemin (1 or 10uM) twice within 7 days. An aliquot of 0.5ml was collected and an equal volume of FBS added at day 7 and frozen at -70°C until assayed. For the HTLV-IIIB AZT-sensitive HIV, the 50% inhibition concentration (IC_{50}) of AZT was 0.008uM (Fig. 1). Hemin alone at a concentration of 10uM, almost completely blocked replication (Fig. 1). The addition of hemin with AZT reduced the IC_{50} of the drug markedly (Fig. 1); because of the efficacy of hemin alone at the 10uM dose, augmentation of the virucidal effect of AZT could not be determined.

In the AZT-resistant HIV strain from patient 2, the IC_{50} of AZT alone in the H9 cells exceeded 1uM. Hemin alone at 1uM had no effect on virus replication; at a concentration of 10uM, substantial inhibition (40%) of virus growth was observed (Fig. 2).

The dose-response effect of hemin alone on the replication of HIV in H9 cells of both the AZT-resistant and the AZT-sensitive strains of HIV was also determined. Viral replication of the drug sensitive strain was reduced by 50% at a hemin concentration of <0.05uM (Fig. 3); for the drug-resistant strain the comparable inhibitory concentration of hemin was about 15uM.

These data indicate that hemin (10uM) alone was able to inhibit replication of an AZT-sensitive isolate in cultured PBL and that in combination with AZT, hemin in concentrations of 1uM or 10uM greatly enhanced AZT efficacy against a drug resistant HIV strain in such culture. Against both AZT-sensitive and AZT-resistant viral strains grown in H9 cells, hemin alone displayed virucidal properties and also augmented the antiviral actions of AZT against the AZT-sensitive viral isolates. The doses of hemin required for these actions were markedly smaller than the dose of hemin required to inhibit virus-associated RT activity of Rauscher murine leukemia virus (1), and are significantly less than those used in other mammalian cell systems where they have proved to be non-cytotoxic (8,9).

To determine the effects of a combination of heme and EPO on the adverse effects of AZT, normal human bone marrow hematopoietic colonies were grown in methyl cellulose cultures to establish the effects on erythroid colony growth according to the procedure of Iscove et al (10) with 1.0 u/ml to 6u/ml EPO added (Epo Toyobo, Osaka Japan) for CFU-E and BFU-E. CFU-E growth was scored at 7 days and BFU-E was scored at 14 days. The cultures contained 0.1 um/L AZT and growth was observed with and without added hemin. The results are shown in Fig. 5.

As seen in Fig. 5, EPO alone at all concentrations tested was not able to overcome AZT's cytotoxic effect. In all cultures without hemin, a similar degree of inhibition of CFU-E growth was obtained at all concentrations of Epo, and a greater degree of inhibition of BFU-E growth was obtained at all Epo concentrations greater than 1U/ml. On the other hand, hemin was able to protect CFU-E and BFU-E colony growth against the cytotoxic effect of AZT. Addition of hemin (10 umol/L) to cultures suppressed by AZT (0.1 umol/L) increased CFU-E colony count by up to 279% and BFU-E count by 282%. The maximal stimulatory effect of hemin was in the presence of Epo 1 U/ml for CFU-E and 2 U/ml for BFU-E colony formation.

The preferred compositions of this invention are designed for parenteral administration. The antiviral agent or agents will be in isotonic, aqueous, buffered solution, typically, made isotonic by the addition of sodium chloride, glucose or other standard solute. The

compositions may also contain propylene glycol, sesame oil or other inert excipient. As suggested above, AZT and similar agents may be administered separately by the oral route.

The therapeutic compositions of the invention will contain a heme product and may additionally contain one or more of the compounds useful for the treatment of retroviral infections, especially HIV infections attributable to HIV 1 or HIV 2. The heme products and antiretroviral agents will be present in amounts effective usefully to treat a mammal such as a human afflicted with such infections. Anti-HIV nucleosides such as AZT and DDI are preferred. As shown above, the compositions may also contain EPO.

Useful dosage ranges for the active agents of the invention will vary with the selected agent, the age, weight, general physical condition of the patient, and other factors readily evaluated by the attending physician or veterinarian.

With the heme products of the invention, a typically useful dosage range whether or not another anti-HIV agent such as AZT is present will be from about 0.5 to 50 mg/kg body weight. The dosage range for the anti-HIV agent is of the same order of magnitude usually employed with these drugs although, because of the beneficial contribution of the heme products to the compositions, the concentration of the anti-HIV agent in a particular composition may be less than recommended when the agent is used alone.

To provide for treatments at such dosage levels, various dosage units will be provided. Typical dosage units may contain from 0.25 to 50 mg/kg of heme product whether or not used alone. The concentration of anti-HIV drug will be of the same order of magnitude as when used alone.

For AZT, the dosage level when administered with a hemin product of the invention will be from about 5 to 250 mg/kg body weight. Such levels may be conveniently achieved by providing parenteral dosage units containing from about 1 to 80 mg/kg of AZT.

If the composition of the invention contains EPO, the dosage range will normally be from 50 to 300 mg/kg body weight. Typically dosage units will contain from about 10 to 100 units/kg of EPO.

Heme arginate is preferred over heme for use in this invention because the acid addition salt is more water soluble than heme or hemin which is the form of heme usually utilized. Additionally, it can be administered intravenously without the complications associated with intravenous use of heme. Additionally, it is more stable than heme. Both products are normally from animal sources and must be purified by tedious and expensive procedures to reduce or eliminate undesirable contaminants, and to be certain the product is free of virus or other infectious organisms.

The synthetic hemes are especially preferred for the practice of this invention not only because they are soluble at useful concentrations in the buffered, isotonic, aqueous compositions of the invention, and may be administered by any parenteral route including intravenously, but also for several other reasons. They may be synthesized chemically, are not subject to viral or other contamination if handled with ordinary care and, in contrast to the natural products, may be produced in lyophylized forms which are stable for years.

The compositions of this invention will be prepared by the usual procedures employed for such purposes utilizing standard readily available components. Typically, the pH of the buffered compositions will be from about 7 to 8, preferably 7.4 to 7.5.

TABLE 1

HIV Antigen, P24, pg/ml (% Inhibition)

Treatment	AZT-sensitive	AZT-resistant
AZT-sensitive HIV Isolates		
AZT 0	11800 (0.0)	101000 (0.0)
AZT 1um	42 (100.0)	90000 (11.0)
Hemin 1um	104000 (12.0)	99500 (1.5)
Hemin 10um	35000 (70.0)*	101500 (0.0)
AZT 1um + Hemin 1um	42 (100.0)*	1400 (99.0)*
AZT 1um + Hemin 10um	29 (100.0)*	145 (100.0)*

*p values 0.01 - 0.001

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WHAT IS CLAIMED:

1. A method of treating a retroviral infection in mammals in need of such treatment which comprises parenteral administration of a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts of heme, organometallic porphyrins and mixtures thereof in an amount sufficient to effect such treatment.

2. A method of treating a retroviral infection in humans in need of such treatment which comprises parenteral administration of a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts, organometallic porphyrins and mixtures thereof in an amount sufficient to effect such treatment.

3. A method of treating a retroviral infection in humans in need of such treatment which comprises parenteral administration of a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts, organometallic porphyrins and mixtures thereof together with an anti-HIV drug in amounts sufficient to effect such treatment.

4. A method of treating an HIV infection in humans in need of such treatment which comprises parenteral administration of a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group

consisting of heme, acid addition salts, organometallic porphyrins and mixtures thereof together with AZT in amounts sufficient to effect such treatment.

5. A method of treating an HIV infection in humans in need of such treatment which comprises parenteral administration of a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts, organometallic porphyrins and mixtures thereof together with AZT in amounts sufficient to effect such treatment and a sufficient amount of erythropoietin to reverse the adverse effects of AZT.

6. A method as in claim 2, 3, 4 or 5 wherein the acid addition salt of heme is heme arginate.

7. A method as in claim 2, 3, 4 or 5 wherein the organometallic porphyrin is tin protoporphyrin.

8. A method as in claim 2, 3, 4 or 5 wherein the organometallic porphyrin is tin mesoporphyrin.

9. A method as in claim 2, 3, 4 or 5 wherein the pharmaceutical agent is heme.

10. A pharmaceutical composition useful for treating a retroviral infection in mammals comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid

addition salts of heme, organometallic porphyrins and mixtures thereof together with an anti-retroviral drug in an amount sufficient to effect such treatment.

11. A pharmaceutical composition useful for treating a retroviral infection in humans comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts of heme, organometallic porphyrins and mixtures thereof together with an anti-retroviral drug in an amount sufficient to effect such treatment.

12. A pharmaceutical composition useful for treating an HIV infection in humans comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts of heme, organometallic porphyrins and mixtures thereof together with an anti-HIV drug in amounts sufficient to effect such treatment.

13. A pharmaceutical composition useful for treating an HIV infection in humans comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, acid addition salts of heme, organometallic porphyrins and mixtures thereof together with AZT in amounts sufficient to effect such treatment.

14. A pharmaceutical composition useful for treating an HIV infection in humans comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent

selected from the group consisting of heme, acid addition salts of heme, organometallic porphyrins and mixtures thereof together with an anti-HIV drug and erythropoietin in amounts sufficient to effect such treatment.

15. A pharmaceutical composition useful for treating an HIV infection in humans comprising a buffered, isotonic, aqueous solution containing a pharmaceutical agent selected from the group consisting of heme, amino acid addition salts of heme, organometallic porphyrins and mixtures thereof together with AZT and erythropoietin in amounts sufficient to effect such treatment.

16. A composition as in claims 10, 11, 12, 13, 14 or 15 where the amino acid addition salt of heme is heme arginate.

17. A composition as in claims 10, 11, 12, 13, 14 or 15 where the organometallic porphyrin is tin protoporphyrn.

18. A composition as in claims 10, 11, 12, 13, 14 or 15 where the organometallic porphyrin is tin mesoporphyrin.

19. A composition as in claims 10, 11, 12, 13, 14 or 15 where the pharmaceutical agent is heme.

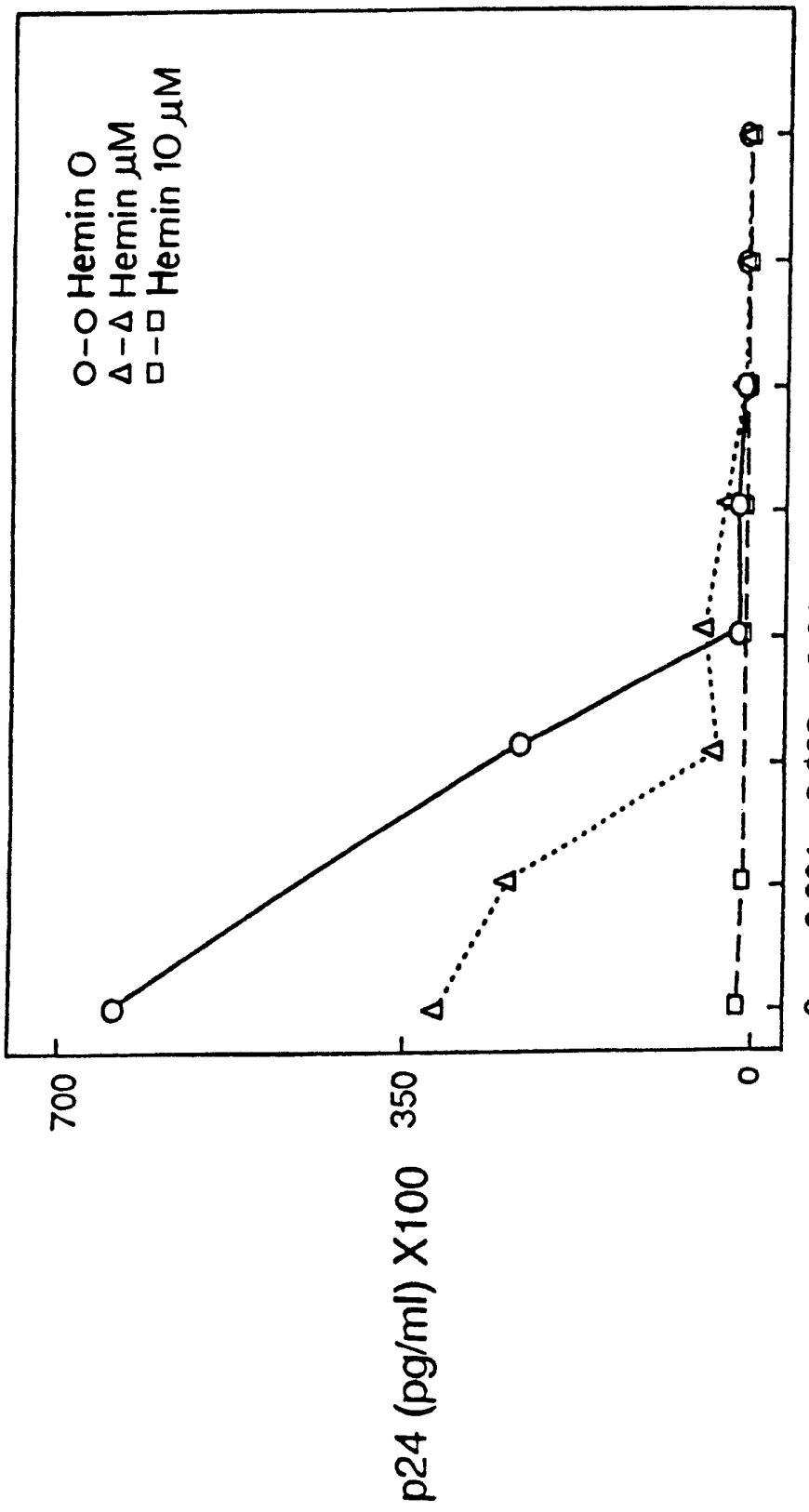
O–O Hemin O
Δ–Δ Hemin μM
□–□ Hemin 10 μM
p24 (pg/ml) X100
700
350
0
0
0.001
0.003
0.01
0.03
0.1
0.3
1.0
AZT (μM)

FIG. I

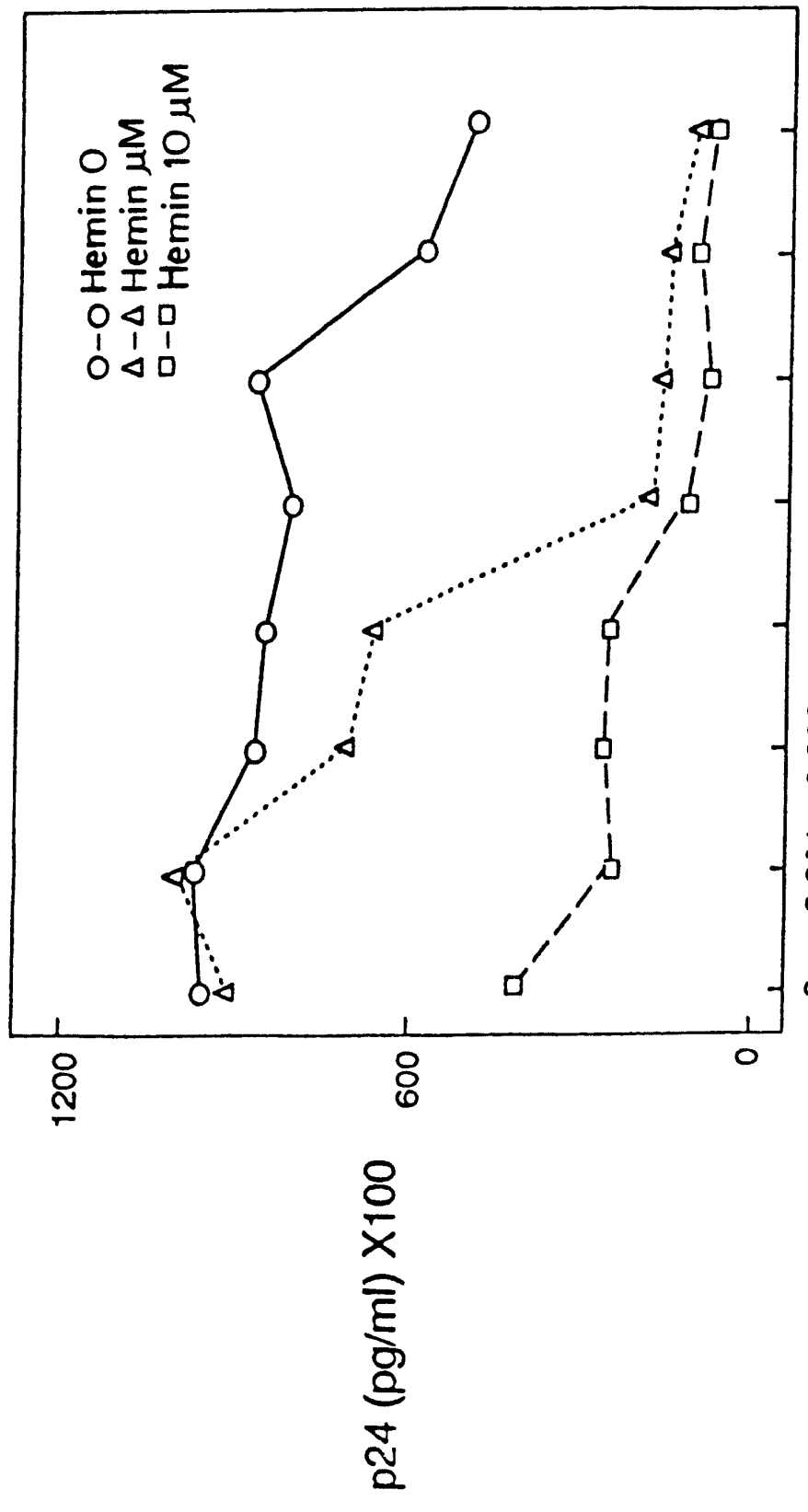
O–O Hemin O
Δ–Δ Hemin μM
□–□ Hemin 10 μM
1200
600
0
p24 (pg/ml) X100
0
0.001
0.003
0.01
0.03
0.1
0.3
1.0
AZT (μM)

FIG.2

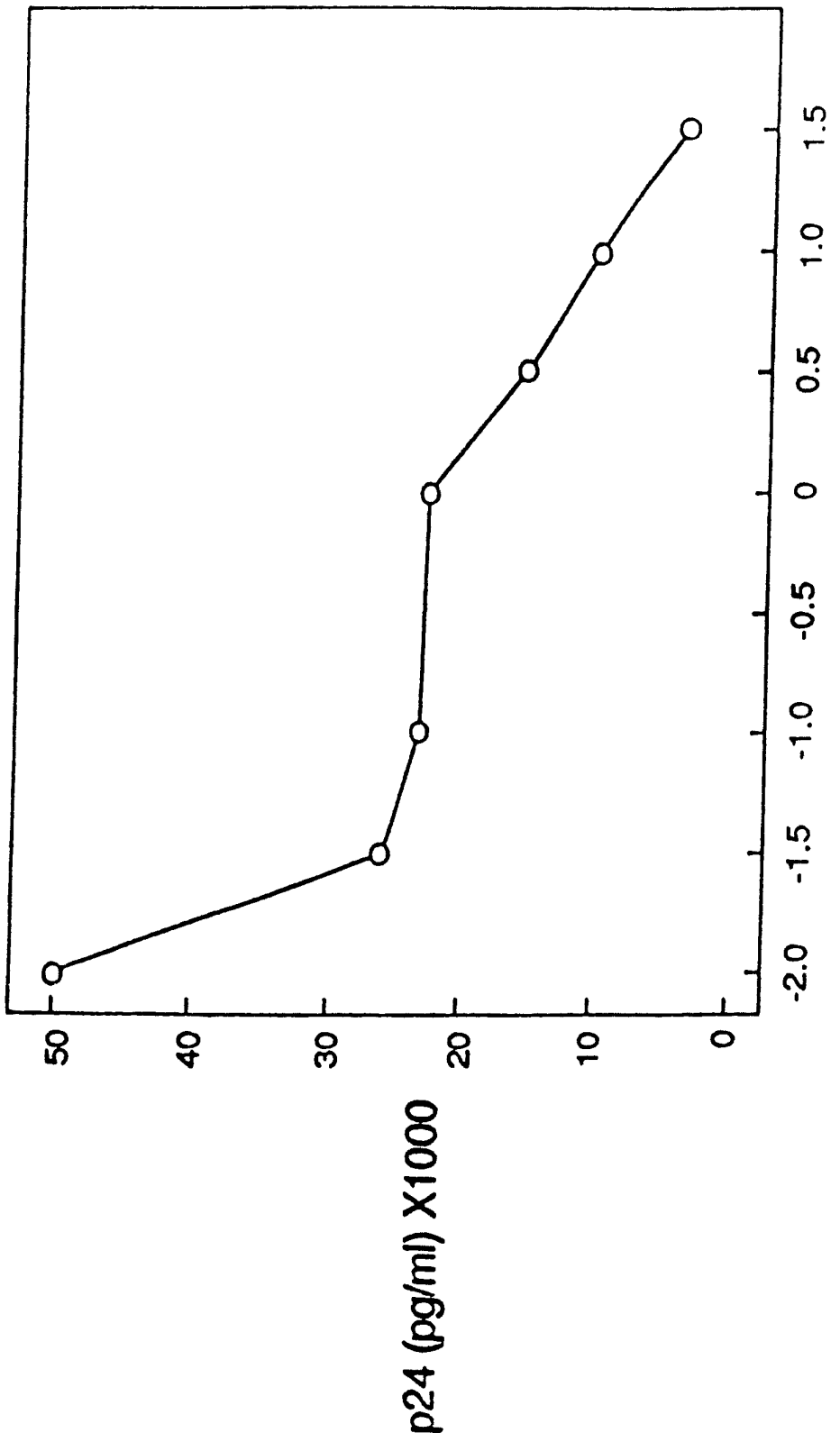
p24 (pg/ml) X1000
50
40
30
20
10
0
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
Hemin (log conc.)

FIG.3

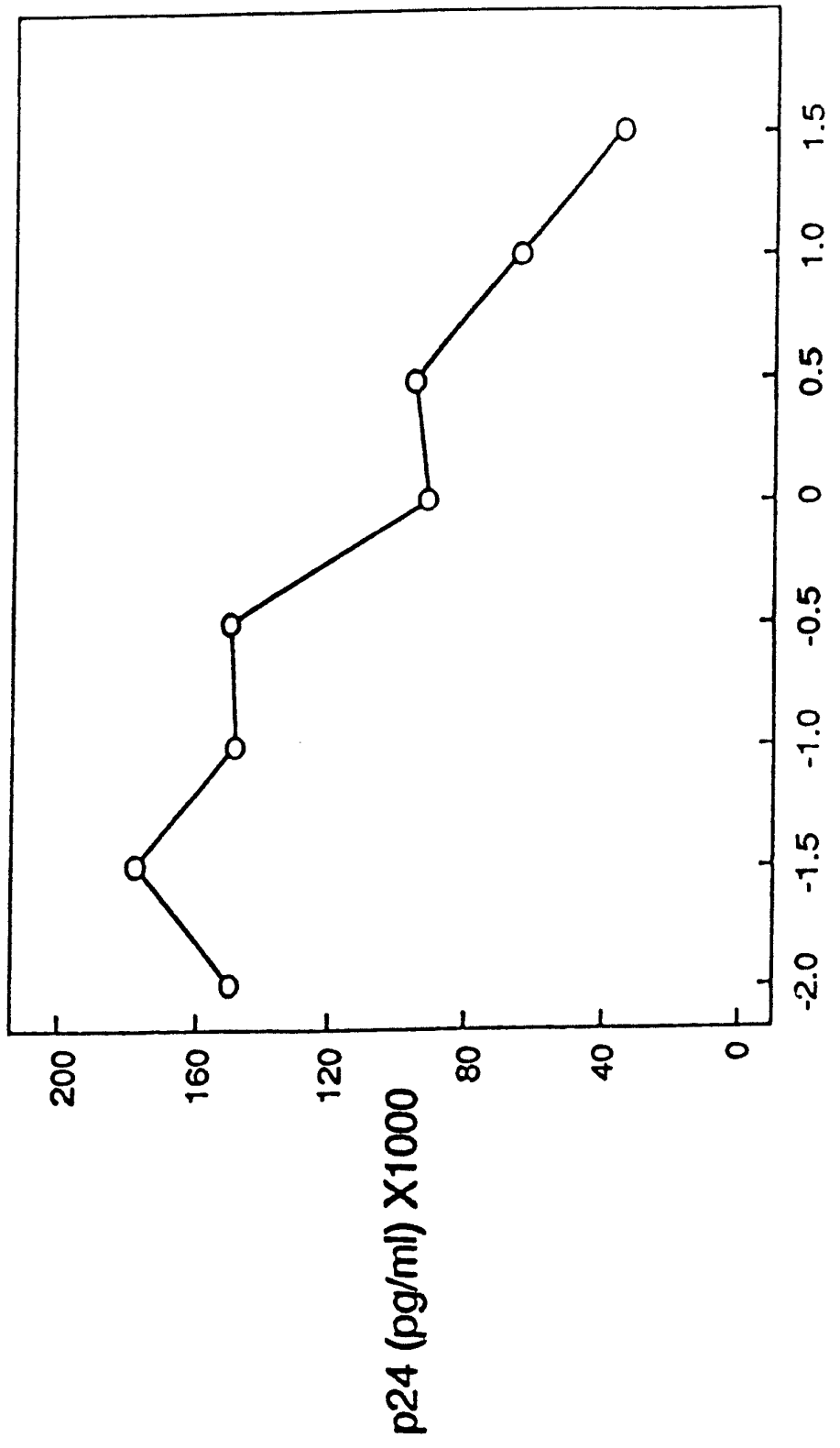
p24 (pg/ml) X1000
200
160
120
80
40
0
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
1.5
Hemin (log conc.)

FIG.4

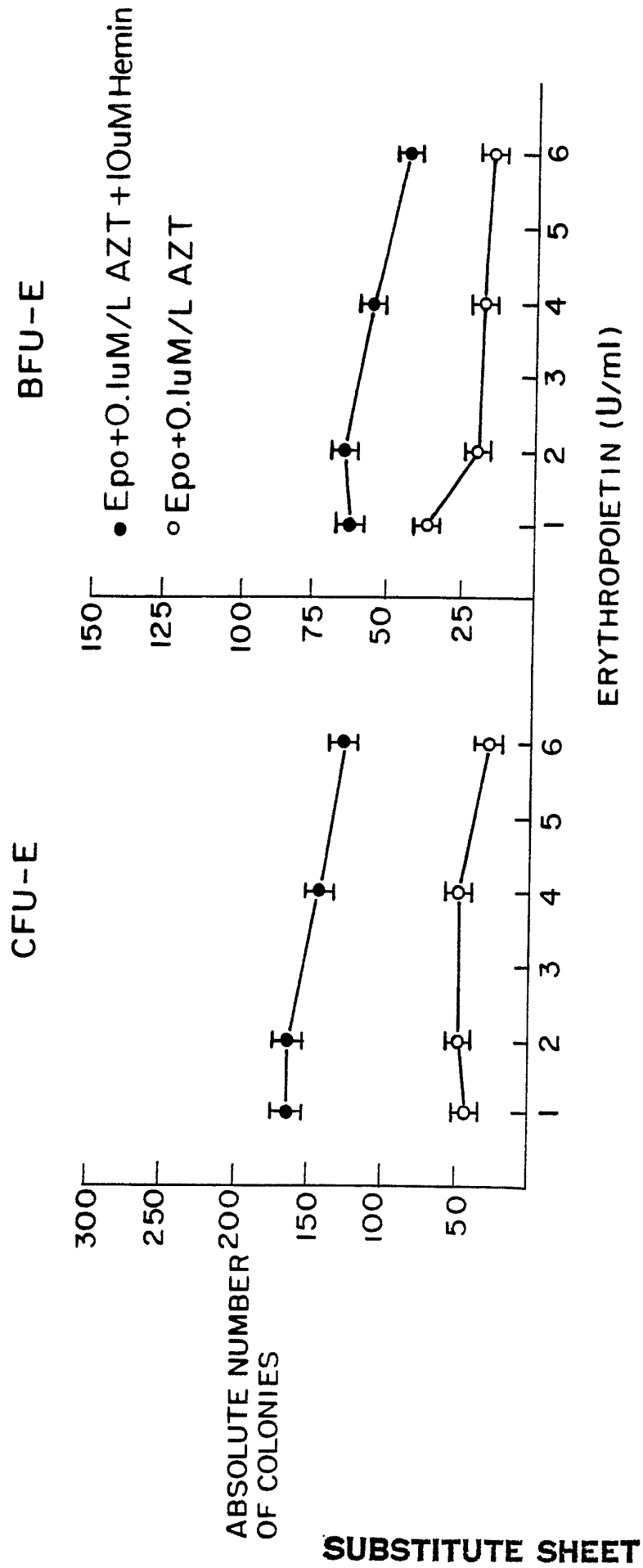
BFU-E
CFU-E
• Epo+O.1uM/L AZT+10uM Hemin
° Epo+O.1uM/L AZT
ABSOLUTE NUMBER OF COLONIES
ERYTHROPOIETIN (U/ml)
150
125
100
75
50
25
300
250
200
150
100
50
1
2
3
4
5
6

FIG.5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/05283

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/10, 31/70, 31/555

U.S.Cl: 514/8, 49, 50, 185, 934

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S. Cl:	514/8, 49, 50, 185, 934

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A,E	US, A, 5,049,493 (Khosla et al) 17 September 1991, see entire document.	1-19
A	J. Med. Chem., Vol 32, no. 3, issued 1989, Chu et al. "Structure activity Relationship of Pyrimidine Nucleosides as antiviral agents for Human Immunodeficiency Virus Type 1 in Peri pheral Blood Mononuclear Cells", pages 612-617.	1-19

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 October 1991	29 NOV 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	John W. Rollins